From:	Shephard, Burt
To:	Anderson-Carnahan, Linda
Subject:	FW: Comparisons of metal concentrations in newest and older shell growth of Port Discovery Seafarms adult Pacific oysters
Date:	Tuesday, July 18, 2017 7:04:11 PM

I meant to add you to the original distribution list. Sorry.

Best regards,

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"Facts are stubborn things, but statistics are more pliable"
- Mark Twain

From: Shephard, Burt
Sent: Tuesday, July 18, 2017 7:03 PM
To: Bachman, Brenda <bachman.brenda@epa.gov>
Subject: Comparisons of metal concentrations in newest and older shell growth of Port Discovery Seafarms adult Pacific oysters

These are the oysters Tom Madsen gave to Margo Young and me on March 22, 2017.

Analytical results for the following metals were below the minimum reporting limit (MRL) for all samples: Sb, As, Be, Cd, Pb, K, Se, Ag, Tl.

Of the remaining metal analytes that are not primary shell constituents (e.g. Ca, Na), four metals most readily lent themselves to statistical comparisons of metal concentrations in the newest shell growth that likely occurred during the period when adult oyster shells exhibited deformations and discoloration: Al, Fe, Mn and Zn. This was based on most or all samples having detected concentrations of these four metals.

EPA has evaluated the concentrations of Al, Fe, Mn and Zn in sections of oyster shells representing the newest, most recent shell deposition that likely occurred during the time when adult oysters exhibited shell deformation and discoloration at Port Discovery Seafarms, and compared the metal concentrations to shell material deposited prior to the onset of toxicity. This analysis was performed

because shellfish, including Pacific oyster, detoxify excess metal concentrations in their water and diet to which they have been exposed by secreting the excess metal into their shells. This detoxification procedure serves to render the excess metals biologically unavailable to elicit toxicity in any of the soft tissues and organs of the oyster.

If oysters had been exposed to one or more elevated metal concentrations in their environment, we expected that exposure to be reflected in elevated metal concentrations in the most recently deposited sections of oyster shell, relative to their concentration in shell sections deposited prior to the onset of observed shell deformation and discoloration. Initial review of the shell analytical data indicated that this metal concentration pattern was observed in most oyster shell samples for Al, Fe, Mn and Zn, with one exception that complicated the statistical analysis. Oyster sample 17120102 showed a concentration pattern opposite to what was expected if oysters had been exposed to elevated metal concentrations in Discovery Bay, i.e. the oldest shell deposits contained the highest metal concentrations, while the most recently deposited shell material contained the lowest metal concentrations. As of the date of this message, EPA has not performed a statistical analysis to determine if the observed pattern of metal concentrations in oyster shell sample 17120102 are statistical outliers compared to the metal concentration pattern in all other oyster shell samples. This possible pattern outlier complicates the statistical analyses described below, and resulted in EPA performing the statistical comparisons both with and without analytical results from sample 17120102.

For aluminum and manganese, the inclusion or exclusion of sample 17120102 from the data sets made no difference in the statistical results. Aluminum and manganese concentrations were not found to be statistically significantly different in newly deposited vs. older shell material, based on results of a Wilcoxon rank sum analysis.

Aluminum – without sample 17120102, $P > 0.2$, no significant difference
Aluminum - including sample 17120102, $P > 0.2$, no significant difference

Manganese – without sample 17120102, $P > 0.2$, no significant difference
Manganese - including sample 17120102, $P > 0.2$, no significant difference

For zinc, the inclusion or exclusion of sample 17120102 from the data sets also made no difference in the statistical results. However, in this case, zinc concentrations were statistically significantly higher in the newly deposited oyster shell material relative to the zinc concentrations deposited in the older shell material in both comparisons.

Zinc – without sample 17120102, $0.02 < P < 0.05$, statistically significant difference
Zinc – including sample 17120102, $0.01 < P < 0.02$, statistically significant difference

For iron, the inclusion or exclusion of sample 17120102 from the data sets resulted in different conclusions from the statistical results.

Iron – without sample 17120102, $0.02 < P < 0.05$, statistically significant difference
Iron - including sample 17120102, $P > 0.2$, no significant difference

Without the results of sample 17120102, iron concentrations in the newly deposited shell material was statistically significantly higher than iron concentrations in the older shell material. When results of sample 17120102 are included in the data sets, no statistically significant differences in iron concentrations between the newly deposited and older shell material were found.

Brenda Bachman asked Katie Adams whether it was possible the analytical results for sample 17120102 and its subsamples had been reversed and reported incorrectly, Katie responded that she did not believe this to be the case. A statistical outlier test on the iron concentration in the subsection of sample 17120102 (subsection "C" in the data report) of 542 mg/kg dw was compared to all other iron results from shell subsections taken from the oldest material deposited in the shell, and was found to be statistically significant (Grubbs' test, $Z = 2.215$, $P < 0.05$. Although the Grubbs test provides evidence that the iron concentration in the oldest part of the shell of sample 17120102 is higher than all other iron concentrations in old shell material, and is a statistical outlier, the Grubbs test does not answer the question needed to determine whether results from sample 17120102 should be included in the statistical comparisons of newly deposited shell metal concentrations to older shell metal concentrations. The question that needs answered is whether the metal concentration pattern in sample 17120102 is different enough than the metal concentration pattern in all other shell samples that it can be considered a statistical outlier. If the metal concentration pattern in sample 17120102 can be identified as a statistical outlier, a basis for excluding it from all subsequent statistical analyses could be made.

Statistical pattern recognition of multiple metal concentration trends in multiple oyster shell analyses requires either discriminant analysis, in which the concentration pattern of each sample is assigned to a predefined class, or a cluster analysis, in which samples are assigned to previously unknown or undefined classes or groups. Since the expected metal concentration pattern in oyster shells is known, discriminant analysis would appear to be the appropriate statistical procedure to identify whether the metal concentration pattern in oyster shell sample 17120102 is an outlier compared to the metal concentration pattern in the other oyster shell samples. As of the date of this message, I have not had time to identify the specific type of discriminant analysis most appropriate to this problem, nor to set up and run the discriminant analysis.

In order to obtain the above statistical analyses prior to Linda Anderson-Carnahan's meeting on 7/19/2017 with Congressman Derek Kilmer, the statistical analyses were performed without preliminary evaluations of normality, homogeneity of variances, processing of non-detect concentrations, fitting of distributions to analytes with a mixture of detected and non-detected concentrations to more accurately estimate mean and median metal concentrations, and statistical outlier analyses of the trends in metal concentrations in the most recently deposited shell material compared to the older shell material deposited by oysters prior to the start of the observed toxicity at Port Discovery Seafarms during the summer of 2015 (i.e. no pattern recognition analyses of metal concentrations were performed). As such, the statistical analyses should be considered preliminary, and cannot be the basis for identifying the source(s) of elevated Fe and Zn concentrations to which Port Discovery Seafarms oysters may have been exposed.

Best regards,

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